containing 1% Triton X-100, buffers containing NP-40 as the detergent resulted in up to a 50% decrease in beta5 20S proteasome activity. For 26S proteasome measurements, many labs utilize varying freeze-thawing methods for cell lysis. We found that brief cycles of freeze-thawing at −20°C resulted in higher beta5 26S proteasome activity compared to freezing cells overnight at −80°C or shaking cells vigorously at 4°C for 1 hour. Optimization of cell lysis techniques is important for helping future studies investigate smaller changes in proteasome activity by allowing the same amount of protein to show significantly higher activity. It also allows proteasome activity to be measured using less protein sample. Our results indicate that the type of cell lysis buffer used as well as the procedure used to disrupt cells is important for optimal proteasome activity measurements. This research is partially supported by NIH grant HL096819.

2087-Pos Board B73

Gold Nanoparticle Coupled with Dynamic Light Scattering for Protein Complex Detection and Analysis

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Many intracellular biochemical processes are triggered by the assembly of proteins into macromolecular complexes, providing a means to control the myriad of biochemical processes for the efficient management of vital biological responses. The detection and analysis of protein complexes is extremely important for understanding molecular mechanism of diseases. We herein present a new technology, NanoDLSay™, for protein complex detection and analysis using gold nanoparticles coupled with dynamic light scattering (DLS). Gold nanoparticles are conjugated with antibody to form nanoparticle immunoprobes. Upon binding of the gold nanoparticle immunoprobes with target protein and protein complexes in the sample solution, the nanoparticle size will increase. Such a particle size increase can be readily detected by DLS and used to extract information on protein-protein interaction and protein complex binding partners. Using this technology, we recently discovered a novel protein complex formed between EGFR, Src and Stat3 protein in the nucleus of a cancer cell line, *Panc-1*. This is a novel finding with potential major clinical implications. NanoDLSay™ is a label-free and solution-based biomolecular assay. Other important applications of NanoDLSay™ as a general tool for biomolecular research will be discussed briefly as well in this presentation.

2088-Pos Board B74

Studying Rapidly Reversible Protein-Protein Interactions by Sedimentation Velocity Analytical Ultracentrifugation

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Studies on protein-protein interactions are of considerable interest in the fields of macromolecular recognition, signal transduction and cellular regulation. With the introduction of modern instrumentation and computational methods, sedimentation velocity (SV) analytical ultracentrifugation has reemerged in the last decade as a powerful technique for characterizing binding equilibria. For rapidly reversible systems of interacting macromolecules when the lifetime of a complex is short relative to the transport time of a SV experiment, complicated transport patterns arise even for simple bimolecular reactions, when all species migrate at different velocities in the solution. Their physical origin of these patterns has been poorly understood, and this has limited fully exploiting all aspects of SV for rapid protein interactions. Recently, we have reported new solutions (effective particle theory, EPT) to the transport equations for rapidly reacting systems, which describe the average sedimentation coefficients and the composition of undisturbed and reaction boundaries with simple analytical expressions and provide a physical picture of the phenomenon of combined transport and reacting processes. In this work, we apply EPT to characterize several protein-protein interactions and demonstrate how the prediction of the transport patterns helps to quantify the assembly energetic interacting systems.

Key words: protein interactions; transport; sedimentation; signal transduction

2089-Pos Board B75

Quantitative Study of Membrane Protein Self-Assembly Using Cell-Free Expression

jerome chalmeau.

We have developed a quantitative method, based on cell-free expression, to study in real-time the self-assembly of membrane proteins (MPs) in vitro. An E. coli transcription/translation extract is used to express MPs either in phospholipid vesicles or on supported phospholipid bilayers. The MPs self-assembly process is studied by fluorescence microscopy and with a quartz crystal microbalance with dissipation (QCMD).

This new approach, which links the information flow to the self-assembly process, was first used to study α-hemolysin, a pore-forming protein produced by Staphylococcus aureus. Two different clones, one labelled with eYFP and the other one with eCFP, were expressed simultaneously inside synthetic phospholipid vesicles. Self-assembly of the heptamers was studied by Forster resonance energy transfer (FRET) between the two fluorophores. In addition, a QCMD was used to study the pore formation in a supported phospholipid bilayer. The cell-free reaction producing the toxin was directly incubated inside the QCMD chamber on the sensor while both frequency and dissipation signals were recorded. The kinetic constant of adsorption was determined.

We are now using this method to study the basal body of the E. coli flagellum, a complex multiprotein nanostructure formed in vivo at the inner membrane. Results for the flagellar proteins FliF and FliG will be presented.

2090-Pos Board B76

Conformation and Self-Assembly of the Transmembrane Peptide Gramicidin A: Insights from ion Mobility Spectrometry and Molecular Dynamics

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Gramicidin A which is composed of alternating L- and D-amino acids is a naturally occurring pentadecapeptide from Bacillus brevis known to form monovalent metal ion channels in lipid membranes. The active form is a noncovalently bound dimer. The conformation and self-assembly behavior of gramicidin A highly depends on the solution environment. In this presentation, we report the use of electrospray-ion mobility-mass spectrometry to study the conformation of alkali metals adducts of gramicidin A monomer, as well as the monomerization and conformer interconversion equilibrium of gramicidin A dimer as a function of the solvent. The conformation of gramicidin A monomer vary significantly upon binding different metal adducts. Enhanced sampling molecular dynamics simulations are performed on alkali metals adducts to provide thermodynamics information of different conformers and gain insights of the interaction of different metal ions with the monomer. The kinetics of the monomerization and conformer interconversion processes of dimer in various alcohol solutions (Ethanol, 1-Propanol and, Isobutanol) are monitored by using the ion mobility profile of the monomer and the dimer. The rate constants and the temperature dependence of the rate constants of the monomer compare well with literature values which were obtained by using fluorescence. Furthermore, we found that the water content in the alcohol solution greatly influences the self-assembly process significantly. The role of water in catalyzing the conformer interconversion is being investigated further. Ion mobility spectrometry (IMS) combined with molecular dynamics simulations is a merging technique for conformational analysis of gas-phase low-lying energy level structures of biomolecules. In this study, we will demonstrate that this gas phase technique can also be of utility in studying a solution phase structural dynamics problem.

2091-Pos Board B77

Organization and Thermodynamics of Peptidic Amphiphiles at the Air/Water Interface

Ozge Engin, Mehmet Sayar.

Peptidic oligomers play an essential role as model compounds for identifying key motifs in protein structure formation and protein aggregation. The spontaneous assembly of these molecules leads to a variety of structures ranging from one dimensional aggregates, like ribbons or cylindrical micelles, to highly organized monolayers in the presence of an interface. Here, we present our results from extended molecular dynamics simulations of self-assembly of amphiphilic peptides at the air/water interface. Experimental results show that these molecules with an alternating sequence of hydrophobic and hydrophilic residues, spontaneously form ordered monolayers at the air/water interface adopting a beta-hairpin like structure within the film. Our results reveal that the beta-hairpin structure can be observed both in bulk and at the air/water interface. However, the presence of an interface significantly shortens the folding time and increases the stability of the hairpin, which is mainly maintained by hydrogen bonds. The adsorption free energy of a single beta-hairpin at the air/water interface is highly negative suggesting that the process is favorable. Decomposition of the free energy into its enthalpic and entropic constituents shows that it is favorable in terms of the first contribution, whereas it is unfavorable in terms the second contribution due to geometric confinement of the peptides at the interface. The alternating hydrophobic-hydrophilic residue sequence provides the main driving force for surface adsorption of these molecules, in agreement with our previous results which show that de-solvation of hydrophobic groups is the main driving